

MIB-1 Labeling Index is Very Important Proliferative Factor for Lymphangioma

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ABSTRACT

Our concept for the treatment of lymphangioma is simple resection if it is small and adjacent to the surface of the skin and intralesional injection of OK-432 if it is big. Resection of the mass is performed if its size has reduced after observation, but persisted. The rapid growth of the lymphangioma has been considered to be due to inflammation and invasion of the vessels, but their relationship remains unclear. Our hypothesis is that the Mib1-labeling index of endothelial cells of fast-growing lymphangioma is larger than that of non-proliferating lymphangioma. Mib-1 labeling index shows their ability of proliferation.

In our study, we could show the high potential cells of lymphangioma had high MIB1 index using the immunological technique of the pathology.

Keywords

Lymphangioma, MIB1-index, proliferative factor.

Introduction

Lymphangioma is an irreversible disease that could recur after the lymphoma has been resected. Our concept for the treatment of lymphoma is simple resection if it is small and adjacent to the surface of the skin and intralesional injection of OK-432 if it is big. Resection of the mass is performed if its size has reduced after observation, but persisted. The rapid growth of the lymphoma has been considered to be due to inflammation and invasion of the vessels, but their relationship remains unclear. Our hypothesis is that lymphangioma which grows rapidly and recurs easily has a higher mib-1 labeling index value compared to lymphangioma that does not recur at all.

The mib1-labeling index is an important indicator of proliferative ability in lymphangioma. In our study, we compared with the cases which exist in a surface of the skin has no recurrence and those which treated many times and aimed for studying a difference

between them for the cell ability by protein related endothelium of the lymphoma using the immunological technique of the pathology.

Materials and Methods

A total of 104 cases of lymphangioma from 1996 to 2018 were enrolled in the study, where 44 patients were treated surgically. Of them, 16 patients were included for analysis. We investigated age, sex, a part, the treatment of the patient, histopathological views. The pathological search performed H-E stain; CD31(JC70A, DAKO x60), as the marker of the vascular endothelium [1]; CD34(QBEnd10, DAKO x200), as the positive reaction for the malignant tumor of the lymph duct [2]; D2-40 (Bioss antibodies, x300), as the maker of the lymphangioma [3]. VEGFR-3 (Bioss antibodies, x300), as an evaluation of the lymphatic duct [4]; PROX-1 (EPR19273, ABCAM, x200), as the instruction from the blood vessel to the lymphatic endothelia [5], and Ki- 67 (Atlas antibodies 50 x9) as a marker for cell proliferation that labeled except G0 period [6].

Results

All cases were classified according to the common microcystic lymphatic malformation type, based on the ISSVA 2018 guidelines. The patients were divided into two groups: the resection only (group A) and the post-OK-432 injection (group B) groups. The median age of operation in group A was 5.8 years (range: 1 to 16 years) and 2 months to 2 years old, an average of 11 months median of 6 months to 1 year old; median age in group B was lower than in group A.

Both groups were mainly consisted of boys. Lymphangioma was seen in the face, neck, trunk, and extremities of group A, but only in the trunk and extremities in group B. Group A consisted of 6 cases, and group B consisted of 6; OK-432 was injected one to three times and operative time ranged from 1 to 3 hours (average: 1.9 hours) in group A, and from 1 to 4 hours (average: 1.83 hours) in group B (Table1). Histopathological findings were observed in all cases, showing many cavities, which all looked like thin, expanded lymphatic, which were lined by a single layer of endothelial cells. Immunohistochemical staining methods using CD31, CD34, D2-40, PROX1, VEGFR3, and Ki-67 were applied for both groups. All patients showed positive reactions with CD31, D2-40, VEGFR3, and PROX1 (Figure 1). With respect to CD34, the small cyst was negative, although a part of the big cyst was positive (Figure 2). There were the parts replaced by granulation tissue after the endothelium was removed. The site where the endothelium was removed poorly reacted with D2-40 but did positively with PROX1 and CD34. The lymphatic wall of all patients in-group B (especially those with big cysts) reacted positively with PROX1 up to the veins (Figure 3). Dyeing of the cytoplasm failed remarkably in-group B and only the nuclei remained. Only one case in-group A was positive for CD34 at the lymphatic cell wall endothelium. The size was big (with a diameter of 10 cm) and bleeding into the lymphatic cyst; therefore, the connection between the vein and the

lymphatic cyst is confirmed. In-group B we could detect that the lymphatic duct branched from the vein (Figure 4). We observed that OK-432 could not reach the small cyst in-group B because remarkable elastic fibers and induration were seen in it, but nothing was observed in the small cyst; VEGFR3 could detect all in the lymphatic endothelium. In order to detect cell proliferation, Ki-67 sections were stained for all cases. We determine the cyst sizes as small (1-50 cells), medium (51-100 cells), and large (more than 101 cells) using the endothelial cell count in the one sight since it was difficult to determine whether the diameter was the longest or not. Many large cysts were found in group A; there were no medium-sized ones, and the endothelium of the lymphatic cells was broken, but the small-sized type persisted without a brake and no Ki-67 staining in group B (Table 2).

Discussion

Lymphatic vessel and development of lymphangiomas

There are two major theories on the proliferation of lymphatic capillaries; one is the centripetal theory by Huntington [7] which suggested that lymphatic endothelium fused each other, then became lymphatic cyst, and lymphatic duct formed the vascular system along the veins and opened to the vein secondly, the other theory was centrifugal theory which Sabin et.al [8] suggested that the lymphatic endothelium which sprouted from the vein developed in the lymphatic network distally. Huntington's theory has been in the limelight recently, since the lymphatic endothelium, which did not originate from the vascular endothelium was found, and a different mechanism controlled both the origin and development between the neck and trunk; therefore, the endothelium which formed the collecting duct came from the lymphatic cyst of the neck. The endothelium of the heart was made both from vascular and non-vascular endothelia, using non-vein-derived stem cells. Conversely, it is thought that lymphangiomas is derive from remnants of five primitive lymphatic cysts (bilateral neck,

Table1: Cases.

Case	Age/Sex	OP	HE fibrosis Cyst size	PROX1	D2-40	CD31	CD34	
OK432(+)	1.3/M	1 st	Big small + -	+	+	+	?	
		2 nd	+ -	+	+	+	-	
	1.3/F	1 st	+ -	+	+	+	-	
		2 nd	+ -	+	+	+	+	
3	2.6/F	1 st	+ -	+	~+	+	-	
		2 nd	+ -	+	-	+	-	
4	1.8/M	1 st	+ -	+	-	+	-	
5	1/F	1 st	+ -	+	+		+	
6	0.6/M	1 st	+ -	+	+		-	
		2 nd	+ -	+	+		+granulation	
		3 rd	+ -	+	+	+	-	
		4 th	+ -	+	+	+*	+	+*
OK432(-)	4/M		--		+	+		
		2	17/M		--	+	+	+
	3	3/F		--	+	+		+
	4	5/M		--	+	+~	+	-
	5	3/F		--	+	一部+	+	~+
	6	2.9/M		--	+	+	+	-

Table 2: MIB1 labeling index.

Case	Ok432	Size	MIB1 positive cells					Average	Mb1 index	Recurrence
1	+	Small	0/19	0/20	0/24	0/15	0/36	0/22.8	0	—
1 second time	+	small-big	0/13	0/93	0/85	0/256	0/22	0/93.8	0	—
2	+	Small	1/12	1/45	0/20	0/23	0/15	0.4/23	1.74%	+
2 second time	+	Middle Destruction	0/19	0/62				0/40.5	0	—
3	+	Small	0/14	0/16	0/26	0/21	0/60	0/27.6	0	—
4	+	Middle Destruction	0	0	0	0	0	0	0	—
5	+	Middle	4/96	0/82	1/30	1/98	2/82	1.6/77.6	2%	+
5 second time	+	Small Middle	1/24	1/22	3/83	1/29	3/37	1.8/39	4.6%	Rapid Growth+
5 third time	+	Middle Destruction	1/92	2/88	1/90	4/59	1/39	1.8/73.6	2.45%	—
6	+	Small	3/45	1/35	2/14	0/20	1/27	1.4/28.2	4.96%	
7	—	Small-big	2/212	1/33	2/27	1/10	1/17	1.4/59.8	2.34%	—
8	—	Big	3/252	2/240				2.5/246	1.0%	—
9	—	Big	18/1180	5/531				11.5/855.5	1.34%	—
10	—	Small-big	1/113	1/76	2/36	0/37	0/47	1.6/61.8	2.59%	—
11	—	Very small	0/19	0/10	0/60	0/7	0/105	0/38.2	0	—
12	—	Big	20/119	3/132	8/210	3/172	1/84	7/143.4	4.88%	+

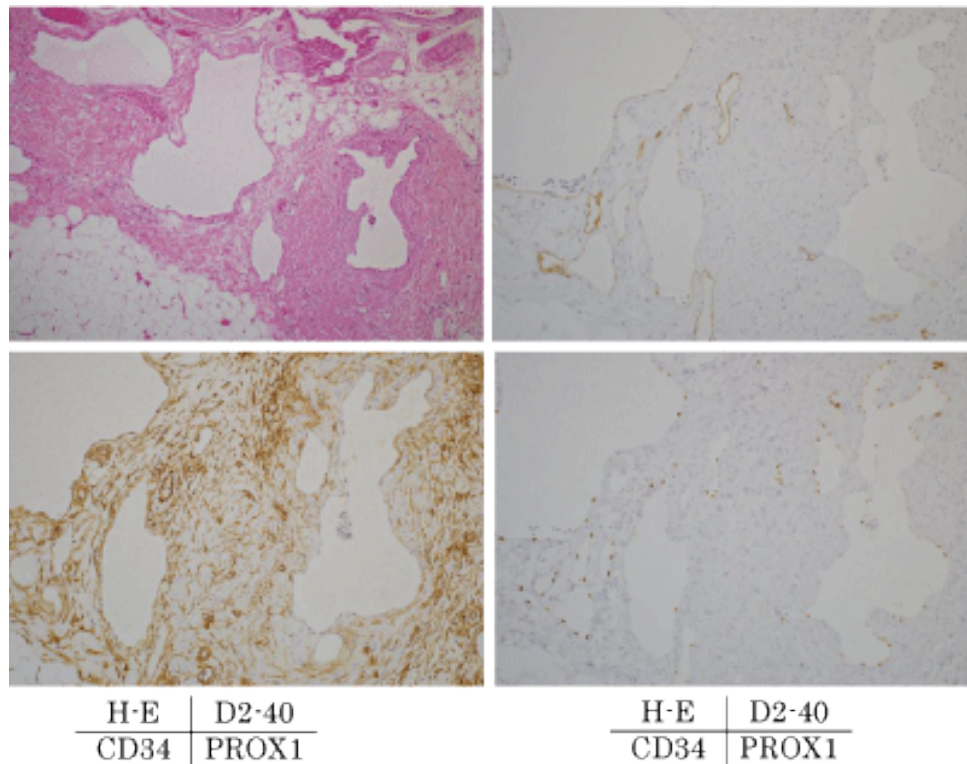
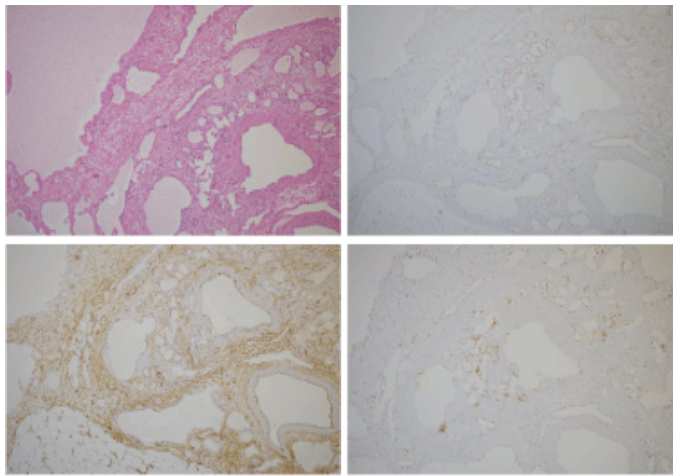


Figure 1: Group A.

PROX1 was all positive and D2-40 is a part negative in the lymphatic endothelial cells. CD34 was negative.



H-E	D2-40
CD34	PROX1

Figure 2: Group B (small cyst).
CD-34 was negative in small cystic lymphatic lumen.

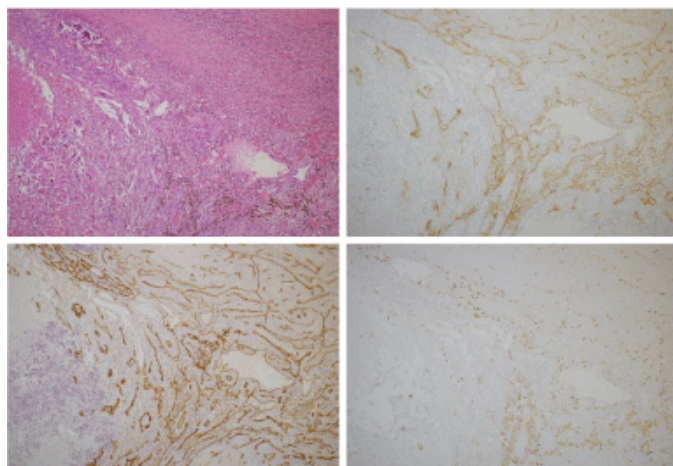
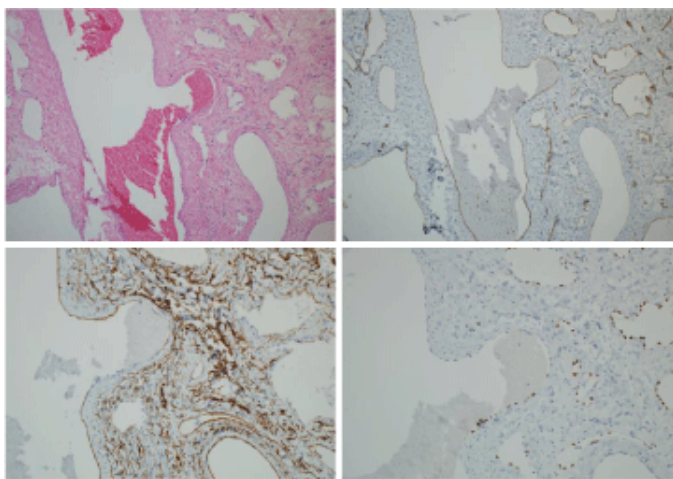


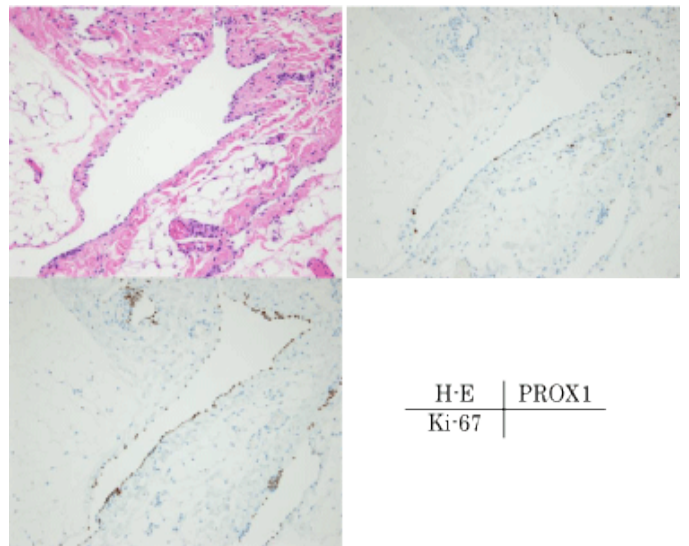
Figure 3: Group B (big cyst).

D2-40 CD34 PROX1 were positive in large cysts and the granulation tissue.



H-E	D2-40
CD34	PROX1

Figure 4: Group B (second operation).
PROX1 was positive in the part which jumped out to the vessel wall.



H-E	PROX1
Ki-67	

Figure 5: Ki-67.

Preparations of PROX-1 and Ki-67 stain were continued to the one of H-E stain.

thoracic duct, retroperitoneum, and posterior part. In this case, the lymphatic cyst couldn't fuse to vein system normally; therefore, a so lymphangioma was formed.

Acquired lymphangiogenesis is different from congenital lymphangiogenesis with respect to the point of sprouting, which is formed from existing lymphatic vessels so that it may differentiate into leaf and a macrophage, converging into a lymph duct. In various inflammation and tissue repair processes, it is reported that VEGF-C, which increases vascularization, and VEGFR3 (which is the receptor of VEGF-C) increased the number of lymphatic vessels. The driving force of lymphangiogenesis is said to be guided by macrophage and VEGF-C, intervened by a thromboxane receptor of the T lymphocytes. Lymphangioma is considered a chronic inflammation without the awareness, since the granulation tissue was observed as pathological findings in-group A. Many small ducts were observed around the big duct of the lymphangioma, but whether these small ones would converge into the big duct or not remains unknown, since no inflammation was found in the small duct. During surgery, we sometimes observed lymph fluid leaking from the small lymphatic vessels; it was hard to see, even though the branch of big lymph vessels were ligated. We cannot exclude the possibility of promotion to change to large lymphatic vessels by secretion of VEGF-C and prostaglandin. It was shown that the possibility of connection between the small and big lymphangiomas was poor, since because the cyst wall of the small lymphangioma did not involve any inflammation, but inflammation occurred in the cyst wall of the big lymphangioma, after the injection of OK-432. The causes which had several treatments showed the lymphangiogenic power in the scar; PROX1 expression was positive, indicating lymphangiogenesis.

Effectiveness of the treatment methods

The normal superficial lymphatic vessel does not accomplish with any vessels, goes through the subcutaneous adipose tissue,

arrives at the regional lymph node in a subcutaneous adipose tissue without the blood vessel. From the surgeons' point of view, it is required to perform complete resection of adipose tissue around the lymph vessel if the lymph vessel in the adipose tissue which cannot be macroscopically observed participates in the recurrence of lymphangioma; our result showed that additional resection of tissue around the lymphangioma was not required; connection between the small and big lymphangiomas was poorer than what we had imagined. A new question is: "how do we determine the possibility of sudden growth of the lymphangioma?" CD34 was reported by Dick in 1997 that there is the stem cell of the malignant tumor and the malignant tumor had positive in CD34.

Rapid growth of the lymphangioma

CD34

Our results showed the positive response of the big duct of lymphangioma and the recurrence rate. We believe that the reason for treatment of a lymphangioma with a high recurrence rate, even though the operation counted several times is difficult, since the cell potential looks the similarity of metastasis of the malignant tumor [9]. Therefore, CD34 is an important index for the potential growth of the lymphangioma.

Ki-67 and MIB-1 labeling index

Cells that are positive for Ki-67 show the intensity of cell activity. In-group B, especially in the cases where only a small cavity remained after the first operation, but the cysts grew. The second operation revealed the destruction of the lymphatic endothelium. It was found that OK-432 was destructive for the lymphatic endothelium; Ki-67 positive cells were removed by OK-432, so recurrence was not observed. In-group A, the big cavity was major, and there were numerous Ki-67 positive cells. This explains why the recurrence rate was low, since all most Ki-67 positive cells were completely removed during surgery. From the result of group A, group B was imagined basically the size of cavity should be big. Yamamoto [10] has classified lymphangioma into two groups; microcystic (where the duct measures under 1 cm) and macrocystic (where it measures over 1 cm); furthermore, it was classified in two types, MLC (large cyst, with a diameter of over 2 cm) and MSC (small cyst, with a diameter of under 5 mm). The LM-MIB index score for the microcystic type was high; MSC had the highest score. Our study could not show the differences of the intensity of cell activity was popular in the small cavity. In one case, injection of OK-432 did not destroy it enough; the middle-sized cyst and Ki-67 positive cells persisted after 3 resection attempts; positive reaction rate decreased and additional resection was not required.

Many reports state the Mib-1 labeling index shows the proliferation of malignant tumors.

Yamamoto et.al [11] also showed that Ki67 positive cell was an important factor to show the effectiveness of R-CHOP method in follicular lymphoma. Absolutely, lymphangioma is not malignant, but some of them characterized by rapid cell proliferation. We can't explain only The MIB-labeling index is a very important proliferative factor for lymphangioma as same as malignant tumors.

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